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The Physiological Basis for Faster Growth in the Sydney Rock Oyster, *Saccostrea commercialis*

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Abstract. Sydney rock oysters were sampled from a mass selection experiment for growth (the "selected" category) and from a control ("not selected") population and held in the laboratory at three ration levels. We evaluated three models to explain faster rates of growth by selected oysters. Selection resulted in oysters feeding at up to twice the rate and with greater metabolic efficiency than controls. A field experiment confirmed that selection leads to faster rates of feeding across a wide range of food concentrations. Selected oysters also grew more efficiently, at a smaller cost of growth (C_g): mean values for C_g were 0.43 $J \cdot J^{-1}$ in selected individuals and 0.81 $J \cdot J^{-1}$ in the controls. In contrast, oysters in both categories showed similar metabolic rates at maintenance, *i.e.*, at a ration supporting zero growth. There was no evidence that differential energy allocation affected the balance between total metabolic requirements above and below zero net energy balance. By experimenting with selected and control oysters of different sizes and ages, then standardizing the data for size, we found no effects of age on the differences due to selection. Faster-growing oysters feed more rapidly; invest more energy per joule ingested; show a higher net growth efficiency; and are able to allocate less energy per unit of tissue growth, than slower-growing individuals.

Introduction

The physiological processes that constitute growth are of fundamental interest. A striking feature of growth in nature is its variability amongst individuals, which is a result of the effects of exogenous and endogenous factors. Of the various endogenous factors involved, genotypic composition may play a significant part.

Genetic properties may affect growth in various ways (Koehn, 1991), including correlations between growth rate and genetic heterozygosity (Mitton and Grant, 1984; Zouros *et al.*, 1988; Britten, 1996). For marine bivalve molluscs in particular, presumed interactions between genotype and growth are of particular interest in aquaculture (Newkirk, 1980), and the selective breeding of oysters has often succeeded in increasing average rates of growth (see review by Sheridan, 1997).

Analysis of the bioenergetics of growth is useful in studies seeking to link phenotypic variability in growth to genetic causes. This approach involves the dissection of growth into its component processes, as represented by the "balanced energy equation" of Winberg (1956; see review by Wieser, 1994). For example, Present and Conover (1992) have described how genetically based latitudinal differences in the growth rate of the fish *Menidia menidia* were due to differences in both food consumption rates and somatic growth efficiency. In this present study, we set out to identify physiological mechanisms to explain observed variability in growth in oysters. We postulated three ways by which an individual animal may increase its rate of growth above that of other individuals, when held in the same environmental conditions. Though not fully independent nor mutually exclusive, these are sufficiently different in both underlying mechanisms and likely ecological consequences to act as useful alternative models to explain variability in growth rate among individuals. We then evaluated these models by comparing oysters artificially selected for faster growth with control, "not-selected," oysters.

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First, in the *increased acquisition* model, an individual may obtain more food per unit time by feeding more rapidly than others, so increasing its metabolizable energy intake (Present and Conover, 1992; Rist *et al.*, 1997; De Moed *et al.*, 1998). This may be evaluated by comparing a variety of traits of feeding behavior (Bayne *et al.*, 1999) and relating the results to rates of growth. We tested the hypothesis that, under similar conditions of ration (quantity and quality), oysters selected for faster growth will have faster rates of ingestion than do control (not-selected) oysters.

Second, in the *modified allocation* model, faster growth may be the result of greater proportional allocation of energy to growth at the expense of other energy-demanding processes, such as body maintenance (Wieser, 1989). We evaluated one aspect of this model by estimating the metabolic rate of selected and not-selected oysters at maintenance, when growth is neither negative nor positive, to test the hypothesis that selected oysters would show reduced maintenance rates.

Finally, in the *metabolic efficiency* model, faster growth may result from a higher growth efficiency (Present and Conover, 1992) from reduced metabolic costs of growth (Wieser, 1994), or from a combination of the two. This model was evaluated in two ways. Firstly, net somatic growth efficiency, defined as the proportion of metabolizable energy intake allocated to growth, was determined. Secondly, by measuring metabolic rates at different rates of growth, we tested the hypothesis that reduced costs of growth correlate, amongst individuals, with increased growth rate.

We used the Sydney rock oyster, *Saccostrea commercialis* (Iredale and Roughley). The New South Wales Fisheries Research Centre at Port Stephens, Australia, established a mass selection program for these oysters in 1990 (Nell *et al.*, 1996). Four selected lines were established for faster growth, and these have been bred in alternate years since. After one generation of selection, Nell *et al.*, (1996) found that oysters from two of the lines were heavier than oysters from two control lines. After two generations, their weight was 18% greater than controls (Nell *et al.*, 1998). Oysters from the third generation of selection (referred to in Nell *et al.*, 1998, as the "loose 2" selection line) were used in the present study and compared with control oysters. The experiment was designed to test hypotheses derived from the three models discussed above and to identify the physiological characteristics that may explain enhanced growth in the selected lines.

Materials and Methods

Material and general procedures

Oysters were provided by the NSW Fisheries Laboratory at Port Stephens. The selected oysters were as described above. Controls were from a commercial oyster farm and are referred to as "not-selected." These oysters were grown under identical conditions to the selected oysters, within Nelson Bay, New South Wales, though they were from a natural larval settlement and not cultured as larvae within the hatchery, as were the selected individuals. Within each category, we distinguished one group of "small" and one group of "large" oysters (Table I). The selected oysters were 23 months old. The ages of the not-selected oysters were not known with certainty, but the "not-selected, small (NSS)" individuals are considered to be of similar age to the "selected, large (SL)" individuals, though of smaller size, and of similar age and size to the "selected, small (SS)" oysters. The "not-selected, large (NSL)" oysters are thought to be about 6 months older than the selected, large oysters.

Table I

Mean (±SD; n = 12) shell heights (cm) and whole weights (shell plus flesh, grams) of oysters in the four experimental categories

Category	Shell height	Whole weight
Selected, Large	7.93 ± 0.33^{a}	52.95 ± 1.05^{c}
Not selected, Large	7.89 ± 0.47^{a}	49.66 ± 2.40^{c}
Selected, Small	6.37 ± 0.32^{b}	38.40 ± 3.87^{d}
Not selected, Small	6.54 ± 0.36^{b}	36.96 ± 2.84^{d}

Values sharing superscripted letters are not significantly different.

Twelve oysters from each experimental category (Table I) were tagged for individual identification and held in the water-table of a research aquarium of recirculating seawater in Sydney. The aquarium contained 600 l of water, of which 33% was replaced every 7 days. Water temperature was controlled at 20 ± 5°C and salinity at 33 ± 1.5‰.

The laboratory experiment was as follows:

20 January to 8 February 1998 (20 days). No supplementary food added; physiological measurements made from 28 January to 5 February and labeled the "field" condition.

9 February to 4 March (24 days). Food added to make up the "middle ration" condition; measurements made from 23 February to 2 March.

5 March to 18 March (14 days). Food added to comprise the "high ration" condition; measurements made 12 to 18 March.

19 March to 9 April (22 days). No food added; the "low ration" condition; measurements made from 6 to 10 April, after 16-20 days without food.

Rations

The food was unicelled algae *Isochrysis galbana* (strain T-ISO) and *Chaetoceros gracilis*, supplied as algal pastes by Reed Mariculture Inc., California. Individual pastes were combined in the proportion 3 parts T-ISO to 7 parts *C.*

gracilis, and the cells were suspended in seawater in a feeding reservoir at the desired concentration (Table II). The cells were dosed to the oysters by peristaltic pump, from 0930 to 1530 daily (middle ration) and 0930 to 1630 (high ration). Cell concentrations in the trays were monitored frequently with a particle size analyzer (Coulter Counter model Z1). Samples of cells from the feeding reservoir were weighed after drying overnight at 80°C, and then measured for nitrogen content (by Leco CHN analyser).

Physiological measurements

All oysters were measured for clearance rate, absorption efficiency, rate of oxygen consumption, and rate of ammonia-nitrogen excretion, at each ration level. Following preliminary studies, care was taken not to use the same individuals in any two measurements without at least 24 h of recovery from the stress of handling.

Clearance rate (CR). Clearance rate is a measure of the volume of water cleared of algal cells per hour. When pseudofeces (that is, material cleared from suspension but not ingested) are not produced (as in this experiment), the rate of ingestion of food is calculated as CR × [food concentration].

Oysters were placed individually in 1-l beakers in water from the feeding trays and left undisturbed in a water bath for 30 min. A beaker without an oyster was used as the blank control. The water was gently aerated using Pasteur pipettes coupled to a compressed air supply. About 600 ml of the water was then siphoned off and replaced with seawater containing algal cells at a concentration equivalent to the experimental ration. A 10-ml sample was taken after 10 min and then at 10-min intervals for a further 40 min. Cell concentrations were measured with a particle size analyzer (Coulter Counter model Z1).

For data analysis, a check was first made for linearity (ln cell concentration plotted over time), then clearance rate as liters per hour was calculated according to Coughlan (1969):

$$CR = ([\ln C_0 - \ln C_1] \cdot \text{Vol}/t) - \text{Blank},$$

where C_0 and C_1 are concentrations of cells at the beginning and end of incubation time t, Vol is the volume of water in the beaker, and Blank is the change in cell concentration in the blank control beaker.

Absorption efficiency (AE). Absorption efficiency measures the efficiency with which ingested organic material is absorbed by the animal. When multiplied by the ingestion rate, AE estimates absorption rate (milligrams of organic matter per hour).

Samples of feces were collected from the beakers in which the oysters were held for CR measurements. It proved impossible to collect enough material for analysis by individual; rather, samples were pooled according to category, at each of the middle and high ration levels. Food cells were sampled at the same time. Samples were filtered onto ashed, preweighed, GF/C filters, washed with 0.9% ammonium formate, dried overnight at 80°C, weighed, ashed for 4 h at 450°C, and weighed again. AE was estimated according to Conover (1966):

$$AE = F - E/[(1 - E) \cdot F],$$

where F and E are the ratios of ash-free dry weight to dry weight of the food and feces, respectively.

Oxygen consumption (VO_2). Oxygen consumption is an indirect measure of the metabolic rate, or rate of energy expenditure, by the animal.

Oysters were placed individually in airtight flasks of ~500 ml volume, on a perforated base that allowed stirring of the water by magnetic stirrers. Each flask was fitted with a Strathkelvin oxygen electrode to record the rate of decline of dissolved oxygen in the flasks. The flasks were also fitted with two 5-ml syringes, one containing algal cells, the other empty as a compensation chamber. After 30 min at constant temperature in a water bath, the algal cells were injected into the flask to achieve a cell concentration equivalent to that measured in the feeding trays (middle and high ration levels only). The rate of oxygen consumption was then recorded for a further 60–90 min. For each set of measurements of five oysters, one flask was used as a blank control.

The rate of oxygen consumption, as milliliters of oxygen per hour, was calculated as:

$$VO_2 = ([O_{2t1} - O_{2t2}] \cdot \text{Vol}/t) - \text{Blank},$$

where O_{2t1} and O_{2t2} are oxygen concentrations (milliliters per liter) at least 30-min apart; Vol is the volume of water in the flask; t is the time in hours, and Blank is the change of oxygen concentration in the blank control respirometer.

Excretion rate (VNH_4–N). This is the rate at which nitrogen is excreted as ammonia. Oysters were placed indi-

Table II

Maximal concentrations of cells (T-ISO + C. gracilis) *and total particulate matter (TPM) in the oyster trays for each of the experimental rations*

Ration	Maximal cell conc:10^3 ml^{-1}	Maximal TPM: mg · l^{-1} [="Peak rations"]	% body weight ingested · d^{-1}
Low	2.9 ± 0.9	0.07 ± 0.02	—
Middle	44.0 ± 1.9	0.59 ± 0.02	0.87 ± 0.37
High	90.9 ± 5.3	1.07 ± 0.07	2.41 ± 1.58

TPM is based on a conversion from cell numbers (10^6 cells = 0.013 ± 0.003 mg dry mass). The "% body weight ingested" is calculated from mean ingestion rates across the four experimental categories, as presented later in the Results section. All values are means ± SD.

vidually in 1 l of filtered seawater and left undisturbed for 3 h. Beakers without oysters served as blank controls. Concentrations of ammonia were measured using the phenol-hypochlorite method of Solorzano (1969); a full set of standards was analyzed for each experimental run. Rates of excretion, as milligrams of ammonia-nitrogen per hour, were calculated as:

$$VNH_4 - N = (Conc_{expt} - Conc_{control}) \cdot Vol/t,$$

where $Conc_{expt}$ and $Conc_{control}$ are ammonia concentrations in experimental and control beakers, respectively, Vol is the volume of water in the beaker, and t is the incubation time (3 h).

Oxygen:nitrogen ratio. The ratio, in molar equivalents, of oxygen consumed to nitrogen excreted serves as an index of catabolic substrate (Bayne and Newell, 1983), and was calculated to evaluate whether the oysters in the different growth and size categories were utilizing different biochemical substrates, a difference which might then explain other observed metabolic differences.

Growth. The 12 oysters in each experimental category were weighed (shell plus flesh) at the beginning (Table 1) and end of the experiment. Growth was calculated by subtraction and related to the period spent at high ration (14 days) to convert to a daily rate. At the end of the experiment, the oysters were shucked and dry flesh weights determined after drying overnight at 80°C.

Due to uncoupling in the growth of shell and tissue in bivalves (Hilbish, 1986; Lewis and Cerrato, 1997) conversions of total weight to weight of tissue, using a constant conversion factor, must be made with caution. For this study, we derived such conversion factors for each individual at the end of the experiment. Given the relatively short duration of the experiment, we considered it appropriate to use these factors to estimate equivalent dry flesh weight at the start, and so to estimate growth also as milligrams of dry tissue per day.

Converting rates to a standard body size

This conversion was based on allometric relationships between dry flesh weight and the measured physiological rates, following Bayne and Newell (1983):

$$V_{stand} = (W_{stand}/W_{meas})^{\beta} \cdot V_{meas},$$

where V_{stand} and W_{stand} are the standardized rate and dry flesh weight, respectively; V_{meas} and W_{meas} are the rate and dry flesh weight as measured; and β is the allometric exponent in the equation describing physiological rate as a function of body size.

Estimates of β were derived for clearance and respiration rates across all experimental categories from the "field condition" measurements. The exponent for excretion rate is based on a separate sample of oysters (Svensson, unpublished). The exponent for growth was determined from rates of growth calculated (see above) for the high ration condition. The values were as follows:

Clearance rate: $\beta = 0.641 \pm 0.113 (n = 32)$
Oxygen consumption rate: $\beta = 0.536 \pm 0.107 (n = 25)$
Excretion rate: $\beta = 0.772 \pm 0.156 (n = 50)$
Growth rate: $\beta = 1.96 \pm 0.58 (n = 45)$.

As measured over all categories ($n = 48$), the mean dry flesh weight of the experimental oysters at the end of the experiment was 0.920 ± 0.243 g. W_{stand} was set at 1.0 g.

Field measurements

Rates of ingestion were measured in the field on two occasions, as a test of the hypothesis that results of the laboratory experiment on feeding rates, and in the context of a model of energy acquisition, would be repeated under the more natural conditions of food availability. Selected and not-selected oysters of similar size (64.0 ± 3.35 g and 62.7 ± 2.86 g whole weight, respectively) were held overnight in wide-mesh bags at the mouth of the Karuah River as it enters the Port Stephens estuary, on 9–10 September and 3–5 November, 1998. This is an area used for cultivating oysters. The selected oysters were from the same mass selection as those used in the laboratory. During the field measurements, water temperatures were 19.1 ± 1.9°C (over both months) and salinities were 28.2 ± 1.9‰ (September) and 32.5 ± 8.3‰ (November). Total particulate matter in suspension was 8.0 ± 2.3 and 29.3 ± 5.8 mg · l^{-1} in September and November, respectively.

The oysters were placed individually in specially designed trays (36 × 16 × 8 cm, with a sill at one end to reduce turbulent flow) at flow rates of 450 ± 15 ml · min^{-1} of water pumped directly from the river. After 1 h all biodeposits were removed from the trays and the oysters left undisturbed for a further 30 or 60 min. Feces and pseudofeces were then collected quantitatively, together with samples of suspended particulate matter, and filtered onto ashed and weighed GF/C filters. The filters were dried overnight at 80°C, weighed, ashed at 450°C for 4 h, and weighed again. The results were used to calculate rates of filtration and ingestion by the "biodeposition" method as described by Iglesias *et al.* (1998) and Bayne *et al.* (1999).

Statistical analysis

The results of the laboratory experiment were analyzed in three stages, using SYSTAT 6.0 (Wilkinson, 1996).

1. For each ration condition the physiological measurements, standardized to an animal size of 1 g dry flesh weight, were analyzed as a two-way ANOVA with "selection" (*i.e.*, selected or not-selected) and "size"

(large or small) as the main effects. In all cases the "selection × size" interaction was not significant.

2. Given the "repeated measures" nature of the experimental design, a different approach was taken to analyze the data across rations. Three groups of four individuals were first selected at random from each category (selection and size). These were then allocated, again at random, to one of the three ration levels. A three-way ANOVA was then done, with "selection," "size," and "ration" as the main effects. Where both "selection" and "ration" showed significant effect, a regression analysis was performed, with ration as the independent variable, to compare oysters from the different categories.
3. Finally, data for each individual oyster over the three ration levels were analyzed by linear regression, and comparisons between categories were made on the basis of the average "within category" values for the slope and intercept in the fitted equations. These regressions were for three data points only, per individual; only those for which the level of significance in the analysis was $P < 0.10$ were used for comparisons.

The results of the measurements in the field were analyzed by two-sample t test with pooled variance.

Results

Laboratory experiment

Ration levels. The cell concentrations in the feeding trays (Table II) were converted to equivalent dry mass using the constant 0.013 mg per 10^6 cells, and to nitrogen content using 5.6% N by weight. "Peak rations" are the levels recorded between 1100 and either 1500 (low and middle ration) or 1600 (high ration), and are the concentrations applied during the physiological measurements (except excretion rates, which were measured in filtered seawater). Peak rations were as follows: low ration, 0.074 ± 0.018; middle ration, 0.593 ± 0.024; high ration, 1.071 ± 0.068 mg · l^{-1}.

Clearance and ingestion rates. These were measured for the middle and high ration levels only (Table III). Differences due to size alone, following standardization to 1 g dry flesh weight, were significant only for ingestion rates at the middle ration. This result indicates that the effects of age on feeding rates were not greatly significant overall. The effects of selection were greater, particularly at high ration, where the selected, large oysters had significantly faster rates of clearance and ingestion than the not-selected, large and not-selected, small oysters.

When the original ingestion rates (*i.e.*, before standardizing them to 1 g dry flesh weight) were converted to percentages of body weight (as dry mass in milligrams), significant differences among categories were evident (Fig. 1; high ration). Selected oysters at both middle and high ration levels had faster relative ingestion rates than the not-selected oysters, with no significant differences between large and small oysters. For example, at the middle ration, SL oysters ingested 1.14 ± 0.47 %bw · d^{-1}, compared with 0.80 ± 0.22 %bw · d^{-1} for NSL individuals; the values for SS and NSS oysters were 0.88 ± 0.44 and 0.68 ± 0.33 %bw · d^{-1}, respectively (see Fig. 1 for the high ration data).

Absorption efficiency. There were no significant differences due either to selection or to size. Values were all between 0.66 and 0.78 (mean 0.71 ± 0.06), with slightly lower values at high than at middle ration ($P < 0.05$).

Rates of oxygen consumption. Differences among categories (Table IVA) were significant ($P < 0.05$) only for size effects at the middle ration, where the VO_2 was higher for the NSL oysters than for the NSS oysters.

Differences between selected, large, and not-selected, large, and between selected, small, and not-selected, small, oysters were not significant at any of the ration levels.

The effects of ration on rates of oxygen consumption, however, were highly significant for all categories, with

Table III

Clearance rates (CR: $l \cdot h^{-1}$) and ingestion rates (IR: $mg \cdot h^{-1}$) for oysters in each of four experimental categories, at two rations

	CR		IR	
Category	Middle ration	High ration	Middle ration	High ration
Selected, Large	2.53 ± 0.28	3.10 ± 0.37	10.36 ± 1.13	23.59 ± 2.74
Not selected, Large	2.12 ± 0.18	2.05 ± 0.29	8.59 ± 0.73	16.42 ± 2.14
Selected, Small	2.07 ± 0.25	3.00 ± 0.34	8.45 ± 1.04	23.54 ± 2.32
Not selected, Small	1.65 ± 0.22	2.01 ± 0.29	6.73 ± 0.88	15.09 ± 2.16
P for selection	<0.01	<0.01	ns	<0.005
P for size	ns	ns	<0.05	ns

Values are means ± SE, for weight-standardized data; $n = 12$ per category. The results of an analysis of variance are shown as the relevant probability, P, comparing selected with not-selected, and large with small oysters; ns signifies $P > 0.05$.

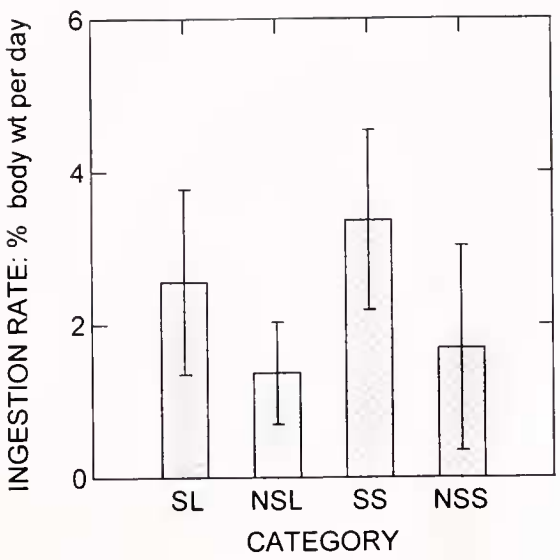

Figure 1. Rates of ingestion (not standardized for differences in body weight), as percent of dry body weight per day, by oysters in the four experimental categories at high ration; means ± SD. The categories are SL, Selected, large; NSL, Not-selected, large; SS, Selected, small; NSS, Not-selected, small.

respiration rate increasing as ingested ration increased. Oxygen consumption rates were converted to energy equivalents as 20.1 J · ml O_2^{-1} (Gnaiger, 1983), and ingestion rates converted as 26.5 J · mg^{-1} (Widdows and Hawkins, 1989). The energy ingested per unit of energy respired was then calculated.

At the middle ration, differences in this efficiency measure between categories were not significant; the overall mean value was 0.83 ± 0.38 joule ingested per joule respired, which indicates a ration level below the maintenance requirement. At the high ration, average energy ingested per unit respiration was higher (1.53 ± 0.55 J · J^{-1}), with significant differences due to selection ($P < 0.01$). Selected oysters (large and small: 1.86 ± 0.78 J · J^{-1}) were more efficient in this respect than the not-selected oysters (1.20 ± 0.55 J · J^{-1}).

This conclusion was confirmed by the analysis of data for individuals. For each oyster, a regression analysis was made of respiratory energy loss, R (J · d^{-1}), as a function of ingested ration, IR (J · d^{-1}). Figure 2 shows the means and standard deviations of the fitted slopes, grouped by category. Categories SL and NSL ($P < 0.02$), and SS and NSS ($P < 0.001$) were significantly different. Differences between size categories (SL *vs.* SS and NSL *vs.* NSS), however, were not significant. On average, selected oysters respired 0.24 J for every joule ingested, across ration levels, compared with 0.45 J by the not-selected oysters.

Excretion rates. At all ration levels, selected oysters excreted more ammonia than the not-selected oysters (Table

Table IV

Metabolic measurements for oysters in four experimental categories at low, middle, and high ration levels

Category	Ration		
	Low ration	Middle ration	High ration
		A. Oxygen consumption rate (VO_2): ml O_2 · h^{-1}*	
Selected, Large	0.408 ± 0.080	0.591 ± 0.138	0.738 ± 0.218
Not selected, Large	0.391 ± 0.105	0.662 ± 0.148	0.701 ± 0.180
Selected, Small	0.363 ± 0.074	0.538 ± 0.126	0.614 ± 0.150
Not selected, small	0.333 ± 0.061	0.514 ± 0.096	0.631 ± 0.110
P for selection	ns	ns	ns
P for size	ns	<0.05	ns
		B. Excretion rate (VNH_4 · N): μg NH_4 · h^{-1}*	
Selected, Large	25.0 ± 4.6	28.7 ± 4.1	27.4 ± 3.4
Not selected, Large	18.5 ± 2.8	19.9 ± 2.7	17.1 ± 2.5
Selected, Small	22.9 ± 4.3	25.4 ± 3.5	31.9 ± 4.6
Not selected, small	8.9 ± 1.2	18.1 ± 2.5	25.2 ± 4.5
P for selection	<0.05	<0.05	<0.05
P for size	ns	ns	ns
		C. Scope for growth (SFG): J · g^{-1} · d^{-1}	
Selected, Large	−212 ± 11	−76 ± 23	87 ± 29
Not selected, Large	−199 ± 15	−154 ± 17	−33 ± 31
Selected, Small	−175 ± 10	−122 ± 28	154 ± 47
Not selected, small	−160 ± 8	−106 ± 25	−33 ± 26
P for selection	ns	ns	<0.001
P for size	ns	ns	ns

Values are means ± SE; $n = 12$ per category. The results of an analysis of variance are shown as the relevant probability, *P*, comparing selected with not-selected, and large with small oysters; ns signifies $P > 0.05$.

* Values represent weight-standardized data.

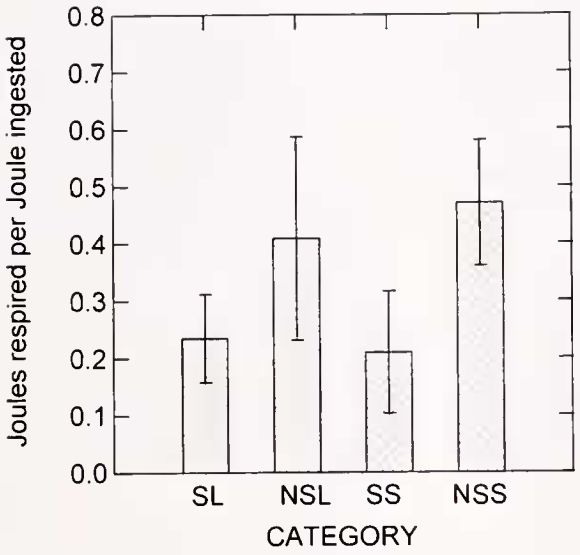

Figure 2. Energy respired ($J \cdot d^{-1}$) per unit of ingested energy ($J \cdot d^{-1}$) by oysters in the four experimental categories; means ± SD. The four categories are SL, Selected, large; NSL, Not-selected, large; SS, Selected, small; NSS, Not-selected, small.

IVB). Size (age) effects were not significant. Only for the small oysters was there a suggestion of excretion rates increasing with increased rates of ingestion.

At the middle ration, excretion rates were fast relative to ingested nitrogen (1.50 ± 1.13 mg excreted · mg^{-1} ingested, across all categories), indicating that this ration was well below the maintenance requirement for nitrogen, as it was also for energy (see above). At high ration, on average, 56% of ingested nitrogen was lost in excretion.

Oxygen:nitrogen ratio. Analysis of variance indicated no significant effects of either growth category or ration on the O:N ratio. Mean ratios for selected and not-selected individuals were 59.8 ± 7.0 and 66.6 ± 7.3, respectively.

Scope for growth (SFG) and maintenance metabolic rate. SFG was calculated as the difference between metabolizable energy intake (ingested ration × absorption efficiency) and the sum of respiratory and excretory energy losses. At low ration, SFG is assumed equal to the summed energy losses (*i.e.*, there was no significant energy intake). There were no significant differences between either selection or size categories at this ration. Similarly, at the middle ration (Table IVC), where the SFG was negative in all cases (*i.e.*, metabolizable energy intake was below the maintenance requirement), there was no significant effect of selection or size overall.

At high ration, however, the SFG was high and positive for selected oysters (both large and small) and negative for not-selected oysters, a highly significant difference (Table IVC; $P < 0.001$). Differences due to size (age) in these weight-standardized data were not statistically significant.

The maintenance metabolic rate (R_{maint}; joules per day) is the rate expressed when growth is neither positive nor negative. This was estimated by plotting R as a function of the SFG (Fig. 3); the intercept at zero growth indicates the metabolic rate at maintenance. Linear regression analysis was applied to all individuals, and the slopes and intercepts were compared by analysis of variance (Table V). Data from eight individuals (2, 3, 2, and 1 in the SL, NSL, SS, and NSS categories, respectively) were rejected as not meeting the chosen level of significance ($P < 0.10$).

There was no significant effect of selection on the estimated maintenance metabolic rate (mean = 308 ± 19 $J \cdot d^{-1}$). Size, however, did have a significant effect; maintenance rate was 337 ± 19 $J \cdot d^{-1}$ for large oysters and 278 ± 19 $J \cdot d^{-1}$ for small oysters.

Regression analysis demonstrated a significant linear relation, over all individual oysters, between intercepts and slopes from the individual regressions of R *vs.* SFG. Therefore, to confirm the absence of any significant differences in estimated R_{maint} due to selection, a separate statistical test was performed. Individual oysters were ranked for R_{maint}, and the two selection categories were compared by the Mann-Whitney U test. The result was not statistically significant, $0.10 > P > 0.05$, over 34 cases.

Growth, the costs of growth, and growth efficiency. Rates of growth, standardized to 1 g dry flesh weight, were derived for the 14 days spent at high ration, from measures of whole weight (shell plus flesh). Growth in selected oysters was faster than in the not-selected oysters (Table V).

The data for growth in whole weight were converted to equivalents in growth of dry tissue weight using total/dry

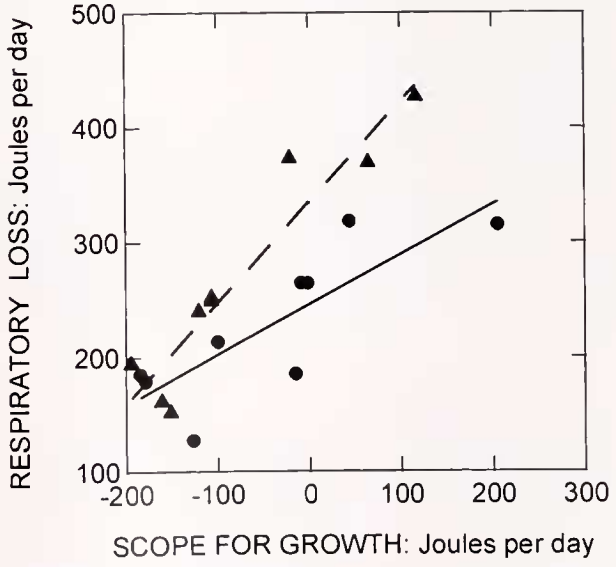

Figure 3. Respiratory energy loss ($J \cdot d^{-1}$) at different levels of scope for growth ($J \cdot d^{-1}$) in three oysters from each of two categories; selected, large (circles) and not-selected, large (triangles). Fitted regression lines are shown. The intercepts at zero scope for growth represent energy losses at maintenance (R_{maint}).

Table V

Rates of growth and growth efficiency at high ration, the estimated costs of growth and maintenance metabolic rate (R_{maint}), for oysters in each of four experimental categories

Category	Growth at high ration: mg total weight · d^{-1}	Growth efficiency	Cost of growth: $J \cdot J^{-1}$	Maintenance metabolic rate: $J \cdot d^{-1}$
Selected, Large	71.4 ± 4.6	0.26 ± 0.03	0.48 ± 0.07	313.1 ± 23.2
Not selected, Large	58.8 ± 6.2	0.16 ± 0.02	0.75 ± 0.14	361.6 ± 25.8
Selected, Small	63.9 ± 5.0	0.29 ± 0.03	0.35 ± 0.07	248.4 ± 16.4
Not selected, Small	52.0 ± 3.7	0.25 ± 0.03	0.85 ± 0.15	308.9 ± 32.2
P for selection	<0.01	<0.05	<0.05	ns
P for size	ns	<0.05	ns	<0.05

Values are means ± SE for $n = 12$ per category (growth and growth efficiency) and $n = 10$, 9, 10, and 11 for categories SL, NSL, SS, and NSS, respectively (costs of growth and R_{maint}). Growth values are for shell + flesh. Growth efficiency is for tissue growth as a proportion of metabolizable energy intake. The costs of growth and maintenance metabolic rate are estimated as described in the text. The results of an analysis of variance are shown as the relevant probability, *P*, comparing selected with not-selected, and large with small oysters; ns signifies $P > 0.05$.

tissue conversion factors for each individual. When values for metabolic rate associated with growth (R_{grow}, in units of joules per day), calculated as $R - R_{maint}$, are plotted against these estimates of tissue growth in energy units, the slope of the regression provides an estimate of the cost of growth, *i.e.*, R_{grow} per unit tissue growth (joules per joule). This analysis yields (see Table V) 0.43 ± 0.19 $J \cdot J^{-1}$ for the selected oysters (categories 1 and 3 together) and 0.81 ± 0.26 $J \cdot J^{-1}$ for the not-selected oysters (categories 2 and 4). These estimates are significantly different ($P < 0.01$).

Growth efficiency was calculated as metabolizable energy intake/tissue growth, both in units of joules per day, for high ration (Table V). The effects of both selection ($P < 0.05$) and size ($P < 0.05$) were significant. Over all categories of selection and size, growth efficiency was low, 0.24 ± 0.04.

Field measurements

Selected oysters ($n = 17$) had significantly faster rates of both filtration and ingestion than not-selected oysters ($n = 21$) on both occasions in the field (September and November; Table VI). Rates were faster in November, when concentrations of suspended particulate material were higher (TPM, 29.3 ± 5.8 mg · l^{-1} compared to 8.0 ± 2.3 in September; particulate organic matter, POM, 4.0 ± 1.5 mg · l^{-1} compared to 1.6 ± 0.4 in September). The ratios of ingestion rates for selected:not-selected oysters were 2.70 for September and 2.06 for November.

Discussion

The rock oysters (*Saccostrea commercialis*) used in this experiment were taken from the third generation of a mass selection program (the "selected" categories) and from a control ("not-selected") population from the same location in the Port Stephens estuary, Australia. At a ration level that peaked daily at 1.1 mg total particulate matter per liter (the "high ration" level), the selected oysters grew, on average, 22% faster than the not-selected oysters. This accords with Newkirk and Haley (1982) for selection for growth in *Ostrea edulis* (23% gain over controls), Paynter and Dimichele (1990) for *Crassostrea virginica* (24%-28% gain), and Toro *et al.* (1994) for *Ostrea chilensis* (13%-33% gain). It also accords with assessment of the Port Stephens selection study itself, where an 18% improvement in growth rate was recorded after two generations (Nell *et al.*, 1998).

The molar ratio of oxygen consumed to nitrogen excreted was calculated to evaluate whether the two groups of selected and not-selected (=control) oysters were catabolizing

Table VI

Filtration and ingestion rates of oysters measured on two occasions in the field at Karuah, New South Wales

	Filtration: mg · h^{-1}		Ingestion: mg · h^{-1}	
Category	September	November	September	November
Selected	20.2 ± 1.6	70.3 ± 17.7	2.7 ± 0.3	16.1 ± 3.0
Not selected	13.3 ± 1.4	22.3 ± 5.4	1.0 ± 0.2	7.8 ± 1.4
P comparing categories,	<0.005	<0.05	<0.001	<0.01

Values are means ± SE for $n = 16$ (September) and $n = 22$ (November). *P* values are the result of *t* tests comparing categories.

different energy substrates at the time of the experiment. Such differences might indicate different physiological states in the two growth categories, which would render more detailed metabolic comparisons complex. There was, however, no significant difference in the O:N ratios ($P > 0.05$), in spite of differences in rates of excretion, suggesting that selection for growth did not shift the normal seasonal pattern of metabolism significantly in these oysters.

Until we know more about nitrogen metabolism in this species, we cannot fully interpret the observed differences in excretion rates. However, the selected oysters both fed more quickly and excreted nitrogen at a higher rate than the control individuals. This is not unexpected, but more information on the relationship between ingested and excreted nitrogen is needed before these observations can be set in context with selection for growth.

We proposed three models to explain the observed differences in rates of growth: faster growing individuals may feed more rapidly; they may reduce their maintenance energy requirement; or they may grow more efficiently than slower growing individuals. The results of the laboratory experiment supported the first and third, but not the second, of these models. Further, by experimenting with different sizes (and therefore ages) of oysters, but correcting the measurements according to observed size/rate relationships, we demonstrated that age was not a significant factor in explaining most of the observed differences between individuals. The exceptions were estimated maintenance metabolic rate, which increased with age, and growth efficiency, which declined with age.

Oysters from the selected line had faster clearance rates (volume of water cleared of food cells per hour) than control, not-selected oysters. This was reflected in a 44% increase in ingestion rates at the greatest ration. Because of a similarity across experimental categories in the efficiency with which these cells were absorbed in the gut, an identical difference in metabolizable energy intake was observed. In experiments with hybrid and inbred lines of Pacific oysters (*Crassostrea gigas*), Bayne *et al.* (1999) recorded faster clearance rates by hybrids in three out of four comparisons, consistent with observed differences in growth rate. Genetically based differences in feeding behavior are an important component of differences in rates of growth among individual oysters.

A similar difference between selected and not-selected oysters was observed in the field experiment, in which a different technique for measuring feeding behavior was used (the biodeposition method in the field, in contrast to direct cell counts in the laboratory), and the concentration of food was significantly greater (8.00 ± 2.28 and 29.3 ± 5.8 in the field, compared with a maximum of 1.07 ± 0.07 mg $\cdot$ l^{-1} in the laboratory). Under these conditions the differences between the two experimental categories of oyster were actually more marked than in the laboratory. This finding lends general support to the "energy acquisition" model and demonstrates that the inferred genetic component of variability in feeding behavior supports faster growth, both under natural circumstances in the field and in the laboratory.

Feeding rates were not only faster in selected oysters, they were also more metabolically efficient. Feeding is not itself energetically expensive in bivalve molluscs, although the total costs of feeding and digestion plus absorption may account for up to 20% of total metabolic rate (Hawkins and Bayne, 1992). Feeding rates may increase significantly without seriously compromising net energy yield. The rock oysters selected for growth achieved a greater gain of energy per unit of energy lost in metabolism than did control oysters. The actual mechanisms of feeding that are responsible for these differences remain unknown. We presume, however, that increasing rate of ingestion as a mechanism for increasing gross energy yield will be limited eventually by decreased gut passage time, which ultimately limits maximum absorption efficiency (Bayne *et al.*, 1989).

Our second model, which we call the energy allocation model, was not supported by the results of the experiment. The estimated rate of metabolism at maintenance varied between 250 and 360 joules per day, standardized for oysters of 1 g dry flesh weight, and it increased with age but not with selection. An average of 308 J $\cdot$ d^{-1} is equivalent to a maintenance requirement for metabolizable energy intake of ~1.4% of body weight per day. This is similar to published values for other bivalves of similar size (reviewed by Bayne and Newell, 1983) and accords with our conclusion that the middle ration level in the laboratory was insufficient to meet the requirements for maintenance of these oysters. Increased maintenance costs with age have commonly been reported for other bivalves (review by Griffiths and Griffiths, 1987).

Studies with blue mussels, *Mytilus edulis*, by Hawkins *et al.* (1986) and Bayne and Hawkins (1997), and with rainbow trout, *Oncorhynchus mykiss*, by McCarthy *et al.* (1994), have demonstrated how reduced rates of protein turnover contribute to reduced metabolic costs and higher rates of growth. These processes appear to be genotype dependent, and they support the concept of differential energy allocation (Wieser, 1989) as a means of increasing growth. In our experiments, differences in maintenance metabolic rate between fast- and slow-growing oysters were not statistically significant. This result merits further research, however. For example, the data (Table V) show a tendency towards higher maintenance metabolic rates in the not-selected oysters, particularly among the smaller size categories, but with high variance. In similar experiments with Pacific oysters, *Crassostrea gigas* (Bayne, in press), we have observed significant differences in R_{maint} among individuals, which correlated with differences in growth rate. The energy allocation model remains a possibility in

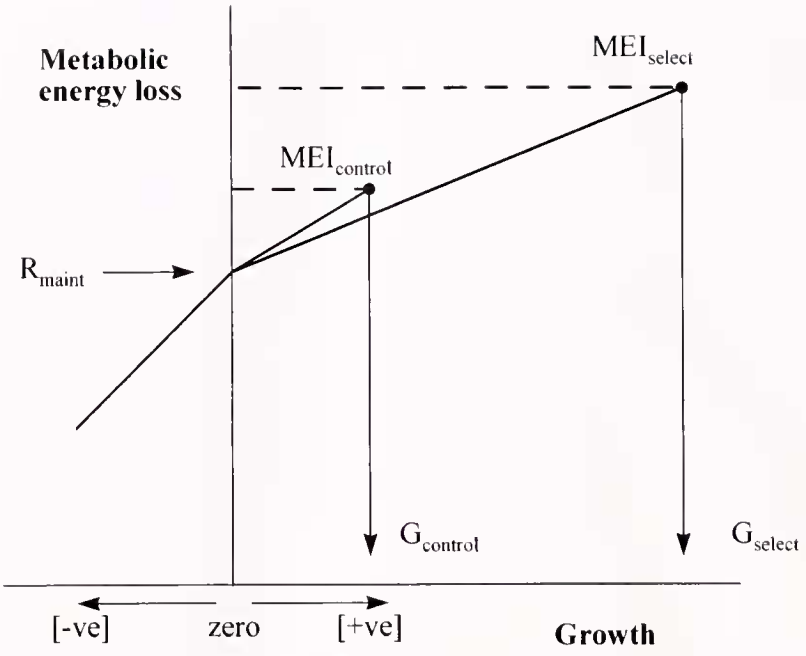

Figure 4. A qualitative illustration of the main findings of this study. Metabolic energy loss is plotted as a function of growth for oysters selected for fast growth, and for control oysters. Below the maintenance requirement, where growth is negative, there is no difference in metabolic expenditure due to selection; the maintenance metabolic demand (R_{maint}) is the same for both experimental categories. However, selected oysters achieve a higher metabolizable energy intake (MEI) than the controls and express a lower cost of growth. The net result is an increased growth rate and a higher growth efficiency (G_{select} *vs.* $G_{control}$). Differences due to selection have been exaggerated for illustration purposes.

the general case, therefore, although not supported directly by these data on *Saccostrea.*

Our third model concerned growth and metabolic efficiency. This was evaluated by estimating both the costs of growth and net growth efficiency in selected and not-selected oysters. The results supported the hypothesis that selected oysters would show a lower cost of growth (0.43 J · J^{-1}) than control oysters (0.81 J · J^{-1}). Both values are high compared with published values (Wieser, 1994; average for "ectothermic metazoans" of 0.30 J · J^{-1}), possibly reflecting a relatively poor-quality diet and slow overall rates of growth. Nevertheless, the differences due to selection, and the lack of significant differences due to age, are evident. Clearly, selection for growth in this species, as in others (Bayne and Hawkins, 1997), involves selection for reduced costs of growth.

Selected and not-selected oysters also differed in growth efficiency measured as the proportion of metabolizable energy intake utilized in tissue growth. This efficiency was low in all cases—for example, 0.28 ± 0.09 and 0.21 ± 0.08 for selected and not-selected oysters, respectively. The results do, however, support the hypothesis that selected oysters utilize a higher proportion of absorbed ration for growth, and do so at a reduced cost of growth relative to the controls.

In summary (Fig. 4), our experiments indicate that mass selection for growth in the rock oyster resulted in individuals that had a greater intake of metabolizable energy by virtue of faster (and more metabolically efficient) feeding, and were able to use this intake more efficiently for growth. Selected and control oysters did not differ in their energetic costs at maintenance. The field experiment confirmed that selected oysters fed more rapidly than the controls. The challenge now is to analyze in more detail the feeding behavior and the metabolic processes that contribute to the costs of growth and to link these processes more directly to observed individual differences in genotype.

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